CHARGE NUMBER: Project 1902

PROJECT TITLE: Tobacco Microbiology

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: D. K. Chadick

DATE OF REPORT: December 6, 1985

I. TEG Solvent Replacement in SEL:

Objective: Evaluate suitable solvents to replace TEG in SEL. The selected solvent should be compatible with the propylparaben preservative and act as a humectant in the finished sheet.

Status: Another flask-level experiment was performed using 1.5% TEG, 1.8% propylene glycol (PG), and 1.8% PG + 1.5% glycerin. The levels of propylparaben were as follows: 100, 200, 300, and 400 ppm. All solvent/humectant percentages were on a (v/v) basis. SEL without class tobacco was prepared in the C Pilot Plant. The SEL was sterilized and inoculated with a 10% (v/v) mixture of the known spoilage bacteria in a 1:1:1:1 ratio. The flasks were incubated four days at 37.5°C at 150 rpm. Three controls were also included in this study, one flask contained only sterile SEL, one contained sterile SEL with a 10% inoculum of the bacterial mixture, and the third was the series of flasks with the solvent/humectants along with a 10% inoculum of the bacterial mixture.

The results from this experiment showed no significant differences in bacteriastasis between the various solvent/humectant and preservative combinations that were tested. The reason(s) for this is not clear at this time. We have observed that the growth characteristics of the four known spoilage bacteria are quite variable in the SEL medium lacking class tobacco and no additional solvents/humectants or preservatives.

Plans: Conduct growth experiments to elucidate the behavior of the control cell cultures. Apply this information to future studies involving solvents/humectants and preservatives.

II. Humectant Replacement Trials in C Pilot Plant:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: Samples of the acceptable sheet are currently being stored in our environmental rooms at 37.5°C and 25°C with 80% RH maintained at both temperatures. The sheet is being analyzed for bacteria, and fungi (yeasts and mold) at 1, 2, 4, 8 and 12 week intervals. The first monthly data is as follows:

TABLE 1

C Pilot Plant RL-TC

Time	Code	Bacterial Colonies/g($\times 10^6$)	Mold Colonies/g
0	1254[1]	19	80
	1255	28	165
	1256	24	80
	Control[2]	2.5	5
4 Weeks	1254	22	15
	1255	20	5
	1256	17	0
	Control	230	0

[1] 1254 - 3% PG + 2.5% Glycerin
1255 - 4% PG
1256 - 4.6% TEG
All test sheets were prepared with 700-800 ppm propylparaben.

[2] The control was Park 500 RL-TC with 4.6% TEG and 700-800 ppm propylparaben.

Note: No preliminary conclusions will be made at this time.

TABLE 2

C Pilot Plant RL-150B

Time	Code	Bacterial Colonies/g($\times 10^6$)	Mold Colonies/g
0	123B[1]	6.3	50
	1264A	5.1	80
	1265B	14	25
	1266C	20	15
	Control[2]	3.0	5
4 Weeks	1263B	18	5
	1264A	6.2	5
	1265B	1.0	5
	1266C	10	5
	Control	9.0	0

[1] 1263B - 3% PG + 2.5% Glycerin
1264A - 4% PG
1265B - 5% PG
1266C - 4.6% TEG
See Table 1 for propylparaben levels.

[2] The control was Park 500 RL-150B with 4.6% TEG and 700-800 ppm propylparaben.

Note: No preliminary conclusions will be made at this time.

Plans: This is an ongoing study and the data will be reported monthly, based on the storage time of the various sheets.

III. Humectant Replacement Trials at Park 500:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: The sheet collection was completed on October 24th, and the samples are being treated as mentioned in item 2. In addition to the storage study done in our environmental rooms, hoghheads of sheet will be stored in a warehouse. At appropriated time, they will be opened and samples will be removed and analyzed as stated in item 2.

Plans: As previously mentioned in item 2.

IV. Humectant Replacement Trials at the RCB Plant

Objective: Store RCB that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: Sheet storage has begun as previously described in item 2.

Plans: As previously mentioned in item 2.

V. Miscellaneous

Objective: Assist members of Analytical Group with an RL sheet storage experiment (3).

Status: Samples of ground tobacco provided by B. Harvey and G. Baker were sterilized and aseptically packed into gelatin capsules. The capsules are currently being stored in one of our incubators at 37.5°C and periodically being sampled for changes in the acetic acid level.

Plans: Assistance in future studies as needed.

References

[1] Chadick, D.; Monthly Report, Project 1902, PM Monthly Summaries, Accession #85-221, 1985, November, 15.

[2] O'Neill, J.J.; Mead, C.A. The parabens: Bacterial adaptation and preservative capacity. J. Soc. Cosmet. Chem. 33: 75-84; 1982.

[3] Baker, P.G.; Harvey, W.R.; The determination of acetic and boric acids in RL sheets by ion chromatography. PM Special Report, Accession #IR85-217: 1985, November, 4.

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